Benthic invertebrate and microbial biodiversity in sub-tropical urban rivers

Correlations with environmental variables and emerging chemicals

Peng, Fengjiao; Pan, Changgui; Zhang, Nai-Sheng; ter Braak, Cajo J.F.; Salvito, Daniel; Selck, Henriette; Ying, Guangguo; van den Brink, Paul

Published in:
Science of the Total Environment

DOI:
10.1016/j.scitotenv.2019.136281

Publication date:
2020

Document Version
Publisher's PDF, also known as Version of record

Citation for published version (APA):
Peng, F., Pan, C., Zhang, N-S., ter Braak, C. J. F., Salvito, D., Selck, H., Ying, G., & van den Brink, P. (2020). Benthic invertebrate and microbial biodiversity in sub-tropical urban rivers: Correlations with environmental variables and emerging chemicals. *Science of the Total Environment*, *709*, [136281]. https://doi.org/10.1016/j.scitotenv.2019.136281

Science of the Total Environment 709 (2020) 136281

Contents lists available at ScienceDirect

Science of the Total Environment

journal homepage: www.elsevier.com/locate/scitotenv

Benthic invertebrate and microbial biodiversity in sub-tropical urban rivers: Correlations with environmental variables and emerging chemicals

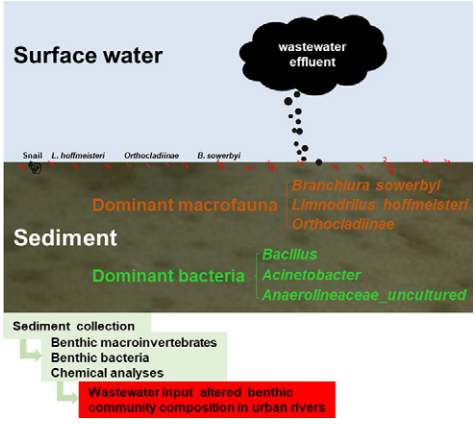
Feng-Jiao Peng [a,b,*], Chang-Gui Pan [c], Nai-Sheng Zhang [a], Cajo J.F. ter Braak [d], Daniel Salvito [e], Henriette Selck [f], Guang-Guo Ying [g], Paul J. Van den Brink [a,h]

[a] Aquatic Ecology and Water Quality Management Group, Wageningen University, P.O. Box 47, 6700 AA Wageningen, the Netherlands
[b] Human Biomonitoring Research Unit, Department of Population Health, Luxembourg Institute of Health, 1 A-B rue Thomas Edison, 1445 Strassen, Luxembourg
[c] School of Marine Sciences, Guangxi University, Nanning 530004, China
[d] Biometris, Wageningen University & Research, P.O. Box 47, 6700 AA Wageningen, the Netherlands
[e] Research Institute for Fragrance Materials, 50 Tice Boulevard, Woodcliff Lake, NJ 07677, USA
[f] Department of Science and Environment, Roskilde University, Universitetsvej 1, Denmark
[g] The Environmental Research Institute, Guangdong Provincial Key Laboratory of Chemical Pollution and Environmental Safety & MOE Key Laboratory of Environmental Theoretical Chemistry, South China Normal University, Guangzhou 510006, China
[h] Wageningen Environmental Research, P.O. Box 47, 6700 AA Wageningen, the Netherlands

HIGHLIGHTS

- A comprehensive biological survey of benthic assemblages was performed in urban rivers.
- Benthic macroinvertebrate- and bacterial community composition shifted along the rural-urban gradient.
- Benthic communities significantly responded to variation in total phosphorus, HHCB and TCS.
- Wastewater input altered benthic community composition in urban rivers.

GRAPHICAL ABSTRACT



ARTICLE INFO

Article history:
Received 4 October 2019
Received in revised form 18 December 2019
Accepted 20 December 2019
Available online 23 December 2019

Editor: Daniel Wunderlin

Keywords:
Urban rivers

ABSTRACT

Urban rivers often function as sinks for various contaminants potentially placing the benthic communities at risk of exposure. We performed a comprehensive biological survey of the benthic macroinvertebrate and bacterial community compositions in six rivers from the suburb to the central urban area of Guangzhou city (South China), and evaluated their correlations with emerging organic contaminants, heavy metals and nutrients. Overall, the benthic macroinvertebrate community shifted from molluscs to oligochaete from the suburban to the central urban rivers that receive treated and untreated sewage. An exception was the site in the Sha River where chironomids were most abundant. The differences in macroinvertebrate community assemblages were significantly associated with chromium, total phosphorus, galaxolide, triclosan and sand content in the sediment. There was no significant difference in benthic macroinvertebrate composition between the dry and wet season.

* Corresponding author at: Aquatic Ecology and Water Quality Management Group, Wageningen University, P.O. Box 47, 6700 AA Wageningen, the Netherlands.
E-mail address: fengjiaopeng@hotmail.com (F.-J. Peng).

https://doi.org/10.1016/j.scitotenv.2019.136281

Double constrained ordination
Benthic macroinvertebrates
Traits
Benthic bacterial community
WWTP effluents

As assessed by double constrained ordination, sexual reproduction was the only trait of benthic macroinvertebrates that showed a significant correlation with pollution variables, as it was significantly positively correlated with chromium and total phosphorus. This suggests that r-strategist occurs in polluted sampling sites. The benthic bacterial community composition showed a significant difference between seasons and among the Liuxi River, Zhujiang River and central urban rivers. The differences in community composition of the benthic bacteria were significantly correlated with galaxolide, total phosphorus, lead and triclosan. These results suggest that input of treated and untreated sewage significantly altered the benthic macroinvertebrate and bacterial community compositions in urban rivers.

1. Introduction

Urban rivers are often exposed to anthropogenic impacts such as wastewater discharge. In the aquatic environment, pathogens, nutrients, heavy metals and organic chemicals can, depending on their physical-chemical properties, bind to suspended particles that eventually settle to the bottom of rivers (Burton, 2002). Consequently, sediments may accumulate contaminants and serve as a reservoir of contaminant bioaccumulation and trophic transfer (Burton, 2002). In highly urbanized areas, such as Guangzhou (South China), wastewater treatment plants (WWTPs) are situated near rivers with effluents routinely discharging into them. Thus, various chemicals such as nutrients, organic and inorganic constituents in the effluents may influence the composition of benthic macroinvertebrate and microbial communities in these rivers (Martino et al., 1998; Li et al., 2016). In particular, emerging contaminants (e.g., active pharmaceutical ingredients, illicit drugs, and personal care product additives) have been considered to be one of the recently identified 12 pressing and emerging threats to freshwater biodiversity (Reid et al., 2019).

Biological assemblages have been used as an effective tool to evaluate contamination caused by sewage (Moreno and Callisto, 2006). For instance, due to their sedentary lifestyle, large size, relatively long life span and variable tolerance to pressures (Jordan and Smith, 2005), benthic macroinvertebrates are frequently used as bio-indicators to evaluate ecological status in the freshwater environment (Rico et al., 2016; Wen et al., 2016). Recently, biological traits analysis (BTA), using a series of functional traits (e.g. feeding habitats, body size, reproduction strategy), has been recognized as a useful analytical approach to describe ecological functioning (Van den Brink et al., 2011; Culp et al., 2011; Bolam and Eggleton, 2014; Pallottini et al., 2017). For example, Munari (2013) reported that biological traits composition of the benthic community was more stable than its taxonomic composition in sandy beach systems affected by coastal development and armouring structures. In comparison with traditionally used approaches (e.g., biodiversity, species abundances), BTA can more reliably indicate human impacts on running waters as biological species trait structure was found to be unconfounded by large spatial gradients (e.g., Statzner and Beche, 2010). However, there are a few weaknesses to BTA, such as traits autocorrelation, traits redundancy and data management (Van den Brink et al., 2011). In the present study, therefore, BTA and taxonomy-based approaches were jointly applied to investigate and characterize correlations between benthic macroinvertebrate community and urban contamination focusing on emerging organic contaminants, heavy metals and nutrients.

In addition to benthic macroinvertebrates, the indigenous benthic bacterial community is another widely used indicator of river environmental conditions (Boothroyd and Stark, 2000). Benthic bacterial communities play a vital role in the transformation and mineralization of inorganic and organic contaminants in wastewater discharges (Li et al., 2016; Atashgahi et al., 2018). In turn, nutrients and contamination can significantly influence the microbial assemblages (Staley et al., 2014; Ibekwe et al., 2016; Wang et al., 2018). For instance, WWTP inputs significantly altered the composition of benthic bacterial communities and decreased their diversity in a highly urbanized river in the Chicago metropolitan region (Drury et al., 2013). In addition, bioturbation activities of benthic macroinvertebrates can also influence the benthic microbial community (Laverock et al., 2010). Recently, it has been demonstrated that the bioturbation by benthic macroinvertebrates can interact with triclosan leading to an increase of its effects on the benthic bacterial community (Peng et al., 2019a). Although benthic macroinvertebrate and microbial assemblages have been individually characterized spatially and temporally in various environments (e.g., Bolam and Eggleton, 2014; Souza et al., 2016; Li et al., 2016; Staley et al., 2014, 2015; Wang et al., 2018), they have rarely been assessed together in field research. However, it is highly important for sediment quality assessment to understand the correlations between contamination and biological assemblages of invertebrates and microbes in sediments.

Given the vital role of benthic macroinvertebrates and microbes in the ecological functioning of rivers, we investigated the benthic macroinvertebrate and bacterial communities at 12 sites located in six rivers in Guangzhou City (South China) from a less densely populated suburban area to a highly urbanized area receiving wastewater effluents. The objectives of this study were (i) to investigate the benthic community composition and spatio-temporal variations, (ii) to reveal the relationship between benthic community composition and environmental variables and emerging chemicals, and (iii) to identify traits that modulate the species-environment relationship. Here we expected that wastewater input would impact the distribution of benthic assemblages, dominant species and traits of benthic macrofauna in receiving rivers. We measured the concentrations of several emerging organic compounds, heavy metals and nutrients in sediments of the sampling sites in both the dry and the wet season (Peng et al., 2017). We used a biological traits analysis (BTA) method in combination with a traditional taxonomy-based method to evaluate the relationships between the environmental and chemical parameters and both communities.

2. Materials and methods

2.1. Study area and sample collection

The study area and sampling locations were the same as reported by Peng et al. (2017). Briefly, the main stream of the Zhujiang River in Guangzhou and its four small tributaries (i.e., Shijing River, Sha River, Liede River and Chebei River) in the central urban area of Guangzhou were selected to represent highly urbanized rivers. Additionally, the Liuxi River, running from the outer suburb to the urbanized area of Guangzhou, was selected to represent a suburban river. The Zhujiang River, Shijing River and Sha River in Guangzhou receive treated effluent from WWTPs (Liu et al., 2017), whereas the Liede River and Chebei River did not receive direct discharges from WWTPs. There are two WWTPs along the Liuxi River, which are much smaller than those in the central urban area (Fig. 1 and Table S1). Seasonal sampling was performed in both the dry (March 2015) and wet season (July 2015) at 12 different sites (S0–S11) along a gradient of increasing population and wastewater impact. Liuxi reservoir (S0) and the upstream part of the Liuxi River (S1) are located in the preserved headwater of Guangzhou city and are minimally exposed to anthropogenic stressors, whereas

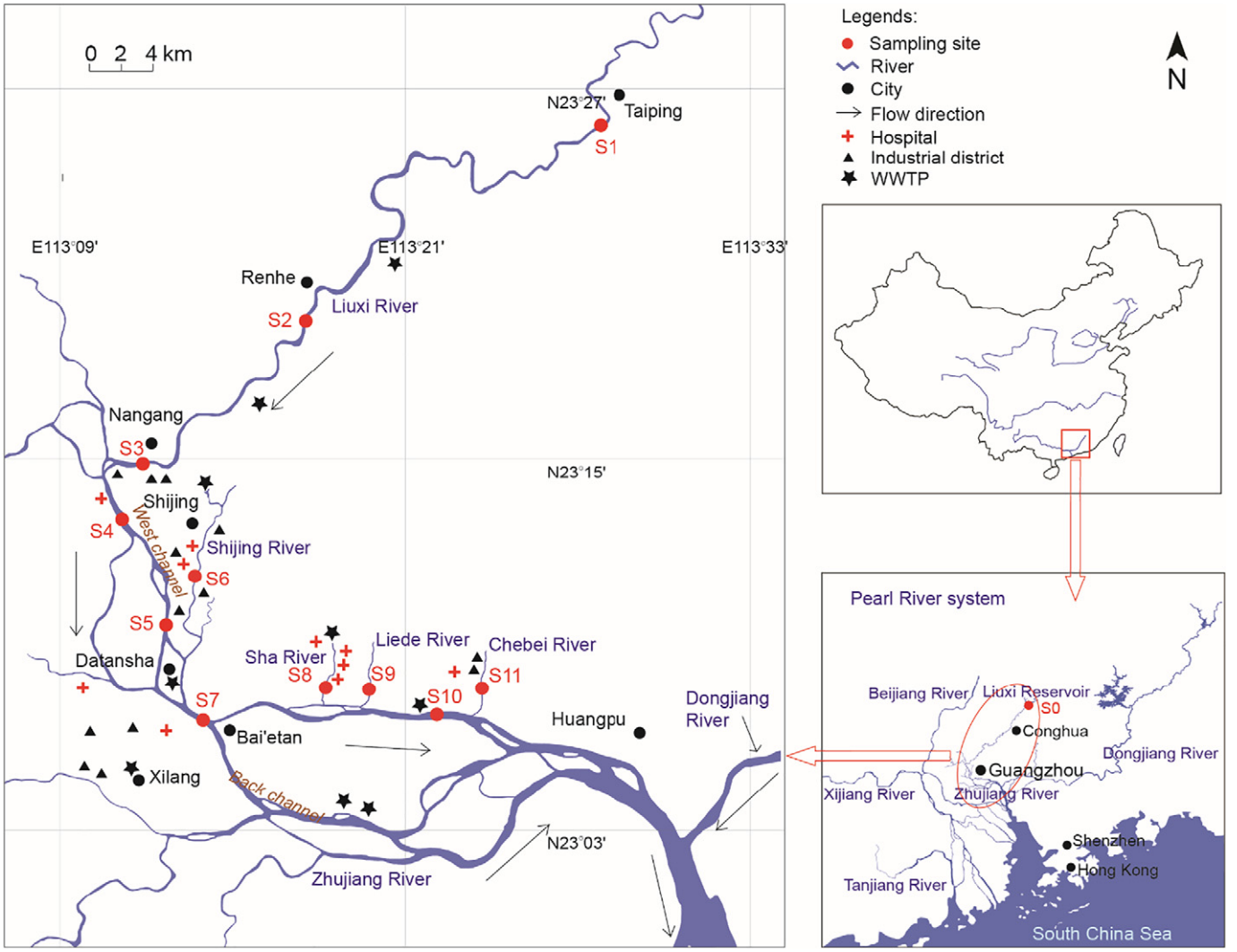


Fig. 1. Map showing sampling locations in urban rivers of Guangzhou in 2015. Specifically, S0 is situated in Liuxi Reservoir that is a drinking water source in Guangzhou. S1, S2 and S3 are located in upper reach, middle reach and lower reach of Liuxi River, respectively; S4, S5, S7 and S10 are in the main stem of Zhujiang River; S6, S8, S9 and S11 are located in Shijing River, Sha River, Liede River and Chebei River in urban region of Guangzhou, respectively.
This map is adapted from our previous work (Peng et al., 2017).

the remaining sampling sites are exposed to urbanization and associated WWTP effluent discharges (Fig. 1 and Table S1). At each site, six random sediments were taken in an area of some square meters with a van Veen grab (area: 0.0435 m^2; volume: 5 L) and pooled for benthic macroinvertebrate analysis. Sediments were immediately wet sieved (300 μm) on site. Benthic macroinvertebrates were then picked out and preserved in 70% ethanol. In the laboratory, the organisms were further sorted and identified to genus level (where possible) under a microscope (Dataset S1). Additional sediment samples were taken for the analysis of bacterial community, organic compounds, heavy metals, nutrients and particle size. The data set on nutrients, sediment particle size as well as emerging organic chemicals from this field survey has been described in Peng et al. (2017).

2.2. Environmental variables analysis

In addition to sediment particle size and the four nutrients (i.e., total organic carbon (TOC), total phosphorus (TP), total nitrogen (TN)), here we also included seven emerging organic compounds (i.e., 4-nonylphenol (4-NP), 4-tert-octylphenol (4-*t*-OP), bisphenol A (BPA), triclocarban (TCC), triclosan (TCS), galaxolide (HHCB) and tonalide (AHTN)) as they prevailed in the sediment from those rivers (Peng et al., 2017). Additionally, eight metals (i.e., arsenic (As), cadmium (Cd), chromium (Cr), copper (Cu), lead (Pb), mercury (Hg), nickel (Ni) and zinc (Zn)) were measured in the sediment samples because of their ubiquitous presence in urban runoff and their potential to impact the environment (Cheung et al., 2003; Liu et al., 2011). The analysis methods used to determine the nutrient, heavy metal and organic chemical concentrations, as well as the method used to determine the sediment particle size, have been described in Peng et al. (2017). The results of these environmental variables were provided in the Supporting information (Dataset S2).

2.3. Traits information collection

The biological traits information of benthic macroinvertebrate species was obtained by matching the monitored benthic macroinvertebrate taxa in this study with the biological trait data available in the Tachet database (Tachet et al., 2000; Usseglio-Polatera et al., 1999, 2000). Since we only focused on their correlations with contamination in the present study, we applied the selection criteria developed by Rico et al. (2016) and excluded traits that are more related to the environmental conditions of the ecosystem, such as current velocity preference. The final trait dataset contained 12 traits, i.e. surface area/volume,

maximal potential size, life cycle duration, potential number of cycles per year, aquatic stages, reproduction, dispersal, resistance forms, respiration, locomotion and substrate relation, food, and feeding habits. These 12 traits further consist of 64 trait states used by Rico et al. (2016) for a European assessment (Dataset S3).

2.4. DNA extraction and amplicon sequencing

Bacterial DNA was extracted from 0.3 g of dry sediment using the E.Z.N.A.® Soil DNA Kit (Omega Bio-tek, Norcross, GA, U.S.). Universal primers 515F 5′-barcode- (GTGCCAGCMGCCGCGG)-3′ and 907R 5′-CCGTCAATTCMTTTRAGTTT-3′ with a unique 8-nt barcode were used to amplify the V4-V5 region of the bacterial 16S ribosomal RNA gene by polymerase chain reaction (PCR). All samples were run in technical triplicate reactions to account for PCR variability. The amplification reactions contained 4 μL of 5 × FastPfu Buffer, 2 μL of 2.5 mM dNTPs, 0.8 μL of each primer (5 μM), 0.4 μL of FastPfu Polymerase, and 10 ng of template DNA. PCR conditions were 95 °C for 2 min for initial denaturation, followed by 25 cycles at 95 °C for 30 s, 55 °C for 30 s, and 72 °C for 30 s and a final extension at 72 °C for 5 min. Amplicons were extracted using 2% agarose gels and purified using AxyPrep DNA Gel Extraction Kit (Axygen Biosciences, Union City, CA, U.S.) according to the manufacturer's instructions. Purified PCR products were further quantified by Qubit®3.0 (Life Invitrogen). The pooled DNA product was used to construct Illumina Pair-End library according to the Illumina's genomic DNA library preparation procedure. After that, the amplicon library was paired-end sequenced on an Illumina HiSeq 2000 platform.

2.5. Data analysis

Significance of the seasonal variation in concentrations of emerging organic compounds, heavy metals, and nutrients were examined using SPSS version 23.0. Prior to analyses, concentration data were examined for normality using Shapiro-Wilk test. If concentration data did not follow a normal distribution, data were log transformed and checked for normality again. While 4-NP, BPA, As, Cr, Pb, Hg, Ni, Zn, TOC, TP, TN and NH_3-N were consequently analysed by the paired samples *t*-test, 4-*t*-OP, TCC, TCS, HHCB, AHTN, Cd and Cu were analysed by the Wilcoxon matched-pairs test. A significance level of $p = 0.05$ was set for comparison between means or rank-sum values.

Spatial and temporal variations in benthic macroinvertebrate assemblages were analysed using Canoco 5.1 software (Ter Braak and Šmilauer, 2018). To test the significance of the difference in benthic macroinvertebrate assemblages among rivers, Monte Carlo permutation tests under the redundancy analysis (RDA) was performed using rivers (i.e., Liuxi River, Zhujiang River and central urban rivers) as explanatory variables and season as covariate. The reverse was done to test the significance of the difference in benthic macroinvertebrate assemblages between seasons. As the expected relationship is monotonic, a partial RDA with forward selection was performed using season as covariates to examine the species-to-environment relationship. Species abundance data were log (x + 1) transformed in all above multivariate analyses to down-weight high abundance values.

Double constrained ordination is an ordination of a rectangular response data table in which the scores of both the rows (samples) and the columns (response variables) are constrained by linear combinations of predictor variables (Ter Braak et al., 2018). In particular, double constrained principal component analysis (dc-PCA) constructs linear combinations of the traits and of the environmental variables that best explain the species data by a bilinear model, thereby summarizing the relationship between traits and environmental variables (Ter Braak et al., 2018). To examine the trait-to-environment relationship, a dc-PCA with forward selection for both environmental variables and traits was performed (using seasons as covariates). Species data (abundance value) were log (x + 1) transformed, to down-weight high abundance values and obtain approximately a normal distribution. The statistical significance of each variable was set at 5% under Monte Carlo simulation tests.

The sequence data were processed using quantitative insights into microbial ecology (QIIME) (version 1.17). Sequences were clustered into operational taxonomic units (OTUs) with 97% similarity cut-off using UPARSE (version 7.1 http://drive5.com/uparse/), and chimeric sequences were identified and removed with Uchime algorithm (Edgar et al., 2011). The phylogenetic affiliation of each 16S rRNA gene sequence was analysed by Ribosomal Database Project classifier (http://rdp.cme.msu.edu/) against the silva (SSU123) 16S rRNA database using confidence threshold of 70% (Amato et al., 2013). The rarefaction analysis was conducted using Mothur v.1.21.1 to reveal the diversity indices including the Chao and Shannon diversity indices (Schloss et al., 2009). The relative abundances of the bacterial 16S rRNA gene at the phylum and genus level were respectively examined to determine the bacterial community assemblages. To detect the influence of wastewater discharge on benthic bacterial community composition, we analysed the differences in the relative abundances of sewer infrastructure-associated bacterial genera (i.e., *Acinetobacter*, *Arcobacter* and *Trichococcus*) (VandeWalle et al., 2012; Newton et al., 2013). Additionally, we also focussed our analyses on denitrifying genera (i.e., *Comamonas*, *Hyphomicrobium*, *Paracocccus*, *Thauera* and *Zoogloea*) that have been found in the activated sludge of WWTPs (Tsai et al., 2012; Wang et al., 2014), and potential bacterial pathogen sequences for assessment of faecal pollution (i.e., *Nocardia*, *Corynebacterium*, *Mycobacterium*, *Bacteroides*, *Arcobacter*, *Aeromonas*, *Legionella*, *Citrobacter*, *Shigella*, *Leptospira*, *Bacillus cereus*, and *Staphylococcus*) suggested by Ibekwe et al. (2016). To display the dissimilarity of bacterial community composition between sampling sites or seasons, a PCA was performed on the OTU data. To test the significance of the difference in benthic bacterial community composition among rivers, a Monte Carlo permutation test under the RDA was performed using rivers as explanatory variables and seasons as covariates, while the reverse was done to test the significance of the difference between seasons. Because there was a significant difference in bacterial OTU composition between seasons and among rivers, bacterial community composition at the phylum level was further compared between seasons and among rivers using partial RDA with seasons or rivers as explanatory variables and covariates, respectively. A partial RDA with forward selection using the environmental variables and emerging chemicals as explanatory variables and season as covariate was performed on the OTU table (relative abundance) to assess the nature and the significance of the relationships. In above multivariate analyses, relative abundance of OTUs and phyla were log (x + 0.0001) transformed, where x represents the relative abundance data.

3. Results

3.1. Environmental variables in the sediment

An overview of the environmental variables in the sediment samples is given in the Supporting information (Dataset S2 and Fig. 2). The concentrations of the selected organic compounds, nutrients and sediment particle size have been reported in Peng et al. (2017). There was no significant difference in organic compound or nutrient levels between the two seasons, except that TCS showed significantly higher levels in the dry than the wet season (paired test, $p < 0.05$). Among the sites (S0-S11), S0 had the lowest sand content (5% in the dry season and 6% in wet season) and the highest clay content (55% in the dry season and 42% in wet season). In contrast, S1 had the highest sand content (79% in the dry season and 70% in wet season) and the lowest clay content (~4% in both seasons). S1 had the lowest silt content as well of ~16% in the dry season and 26% in wet season. There was no clear trend in the sediment particle size for the remaining sites. All the eight heavy metals (As, Cd, Cr, Cu, Hg, Ni, Pb and Zn) were detected in the sediment samples at a wide range of concentrations, with Zn, Cu, Ni, Pb and Cr as

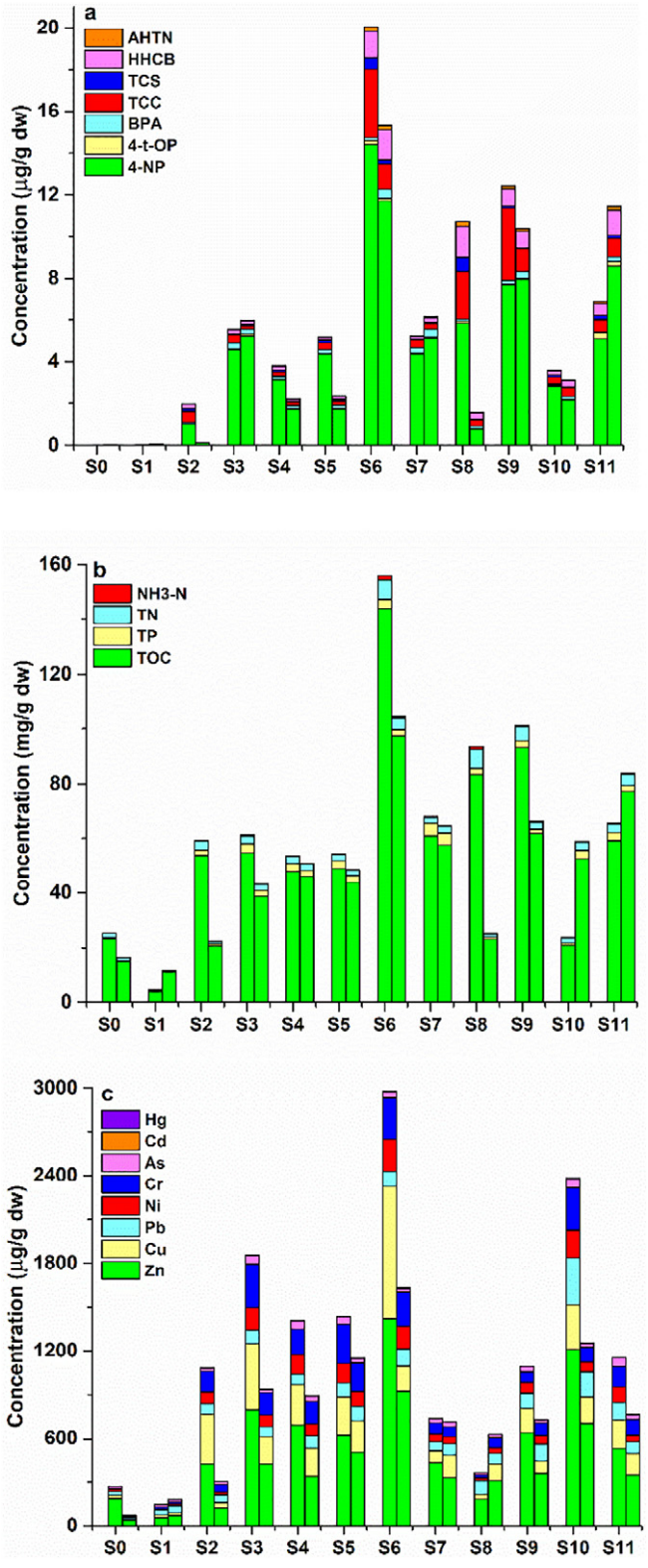


Fig. 2. Concentrations of selected emerging chemicals (a), nutrients (b) and heavy metals (c) in the sediments from the six rivers of Guangzhou in the two seasons. For each sampling site, dry season is on the left side while wet season is on the right side. a) 4-NP, 4-*t*-OP, BPA, TCC, TCS, HHCB, and AHTN are abbreviations of 4-nonylphenol, 4-tert-octylphenol, bisphenol A, triclocarban, triclosan, galaxolide and tonalide, respectively; b) TOC, TP, TN and NH_3-N stand for total organic carbon, total phosphorous, total nitrogen, and ammonia-nitrogen, respectively; c) Zn, Cu, Pb, Ni, Cr, As, Cd and Hg represent zinc, copper, lead, nickel, chromium, arsenic, cadmium and mercury, respectively.

the dominant metals (Dataset S2 and Fig. 2c). Especially, Zn was the most dominant metal in all samples, with concentrations in the range of 189–1420 μg/g dry weight (dw) in the dry season and 38.7–924 μg/g dw in the wet season. Higher concentrations of heavy metals were found at sites in the industrial parts of the Zhujiang River and Shijing River than at the other sites which are located in less industrialised areas of Guangzhou. While As, Cr, Cu, Ni and Zn showed significantly higher concentrations in the dry season than the wet season (paired tests, $p < 0.05$), there was no significant difference in concentrations of Cd, Pb and Hg between the two seasons.

3.2. Benthic community composition and spatio-temporal variation

3.2.1. Benthic macroinvertebrate community

A total of 13 and 12 taxa were identified in the dry and wet season, respectively (Dataset S1). From the upstream part of the Liuxi River (minimally influenced) to the downstream part of the Liuxi River, Zhujiang River and the central urban rivers (influenced by anthropogenic activities), the prevalent benthic macroinvertebrates shifted from gastropoda and bivalvia to annelids *Limnodrilus hoffmeisteri* and *Branchiura sowerbyi* (Dataset S1). However, *Orthocladiinae* was the predominant benthic macroinvertebrate at site S8 (Sha River) in both seasons. Additionally, there were no benthic macroinvertebrates at site S9 (Liede River) in the dry season, and in any sampling sites in the central urban rivers except for site S8 in the wet season (Dataset S1). The benthic macroinvertebrate community composition was significantly different among rivers (Monte Carlo permutation test; $p = 0.002$). There was no significant difference in benthic macroinvertebrate community composition between the two seasons (Monte Carlo permutation test; $p = 0.296$).

3.2.2. Benthic bacterial community

A total of 52 bacterial phyla and 1028 genus were identified in the sediment samples. The five most dominant phyla were *Firmicutes* (9.88–70.1%), *Proteobacteria* (10.2–62.4%), *Actinobacteria* (2.52–31.9%), *Chloroflexi* (1.67–26.5%) and *Bacteroidetes* (0.73–21.8%) (Fig. 3a). In general, *Firmicutes* was the most dominant phylum in most samples, whereas *Proteobacteria* was the most abundant phylum at sites S4 and S8 in the dry season and sites S0, S5 and S8 in the wet season. Additionally, *Actinobacteria* and *Chloroflexi* were the most dominant phylum at site S9 in the dry season and S6 in the wet season, respectively. At the genus level, *Bacillus* (0.11–49.7%), *Acinetobacter* (0.01–45.3%), *Lactococcus* (0.01–20.4%), *Clostridium* sensu stricto *1* (0.36–16.2%) and *Peptostreptococcaceae_unclassified* (0.16–11.1%) were the five most abundant genera (Fig. 3b). *Bacillus* was prevalent at site S3 (downstream part of the Liuxi River) in the wet season, accounting for 49.7% of the bacterial sequences. *Acinetobacter*, *Arcobacter* and *Trichococcus* (WWTPs-associated bacterial genera) showed a higher relative abundance at site S8 than the remaining sites, with *Acinetobacter* as the predominant genus at site S8 in the wet season occupying 45.3% of the bacterial sequences (Table S2). It should be noted that the relative abundance of *Acinetobacter* at site S8 in the wet season was 29 times higher than that in the dry season. Although sites S6, S9 and S11 are also located in the central urban rivers, the abundances of *Acinetobacter*, *Arcobacter* and *Trichococcus* were far lower than site S8 (Table S2). For instance, the relative abundance of *Acinetobacter* at site S8 was 13.5 and 376 times greater than that at site S6 in the dry and wet seasons, respectively. Denitrifying genera showed higher relative abundances in the samples from the central urban rivers as compared with the Liuxi River and Zhujiang River (Table S2). All analysed potential genera for assessment of faecal pollution showed low relative abundance across all samples (0–1.2%), except that *Nocardia* showed a proportion of 11.5% at site S9 in the dry season.

There was a clear separation in bacterial community assemblages at the OTU level between the rivers along the first axis in the PCA ordination diagram: i) samples from the up- and midstream part of the Liuxi

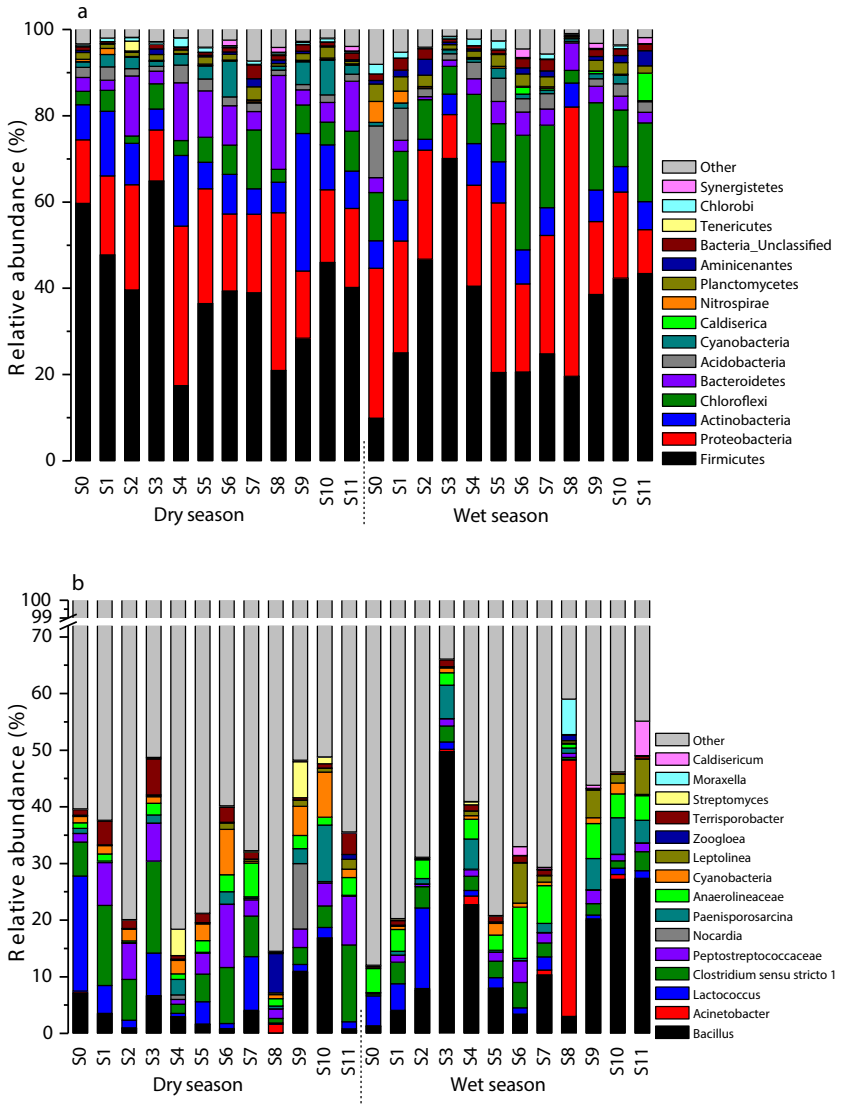


Fig. 3. Relative abundance (%) of the top 15 bacterial phyla (a) and genera (b) in the sediments from the six rivers of Guangzhou in the two seasons.

River (S0-S2) clustered to the right, ii) samples from the downstream part of the Liuxi River (S3) and Zhujiang River (S4, S5, S7 and S10) clustered to the middle, and iii) samples from the central urban rivers (S6, S8, S9 and S11) clustered to the left (Fig. 4). Additionally, there was a significant difference in bacterial community assemblages between the above three groups (Monte Carlo permutation test; $p = 0.002$). There was also a significant difference in bacterial community composition between rivers (Liuxi River, Zhujiang River and central urban rivers) ($p = 0.002$) and seasons ($p = 0.002$). At the phylum level, the bacterial community composition was also significantly different between rivers ($p = 0.002$) and seasons ($p = 0.012$). Among the 10 phyla's showing the most variation in relative abundance among rivers, *Nitrospirae* and *Gemmatimonadetes* showed higher relative abundance in the Liuxi River, *Chlorobi*, *Gracilibacteria* and *WCHB1-60* showed higher relative abundance in the Zhujiang River, and *RsaHF231*, *Lentisphaerae*, *Caldiserica*, *Synergistetes*, and *Cloacimonetes* showed higher relative abundance in the central urban rivers (Fig. S1). Among the 10 phyla's showing the most variation in relative abundance between seasons, only one phylum (i.e., *Candidate division OP3*) was more abundant in the dry season, whereas the remaining nine phyla (e.g., *SHA-109*, *Chloroflexi* and *Aminicenantes*) were more abundant in the wet season (Fig. S2).

3.3. Correlation between benthic community composition and environmental variables

Among the measured environmental variables, Cr, TP, HHCB, TCS and sand content (conditional effects) significantly explained 65% of the total variation in benthic macroinvertebrate community composition (Fig. 5). Cr, TP, HHCB, TCS and sand content respectively explained 24%, 14%, 12%, 11% and 5% of the total variance. Species on the left-hand side of the diagram, such as L. *hoffmeisteri* and *B. sowerbyi*, were positively correlated with Cr and TP concentrations and occurred in higher abundances in the samples from the downstream part of the Liuxi River and Zhujiang River with higher Cr and TP concentrations. In contrast, those on the right-bottom side (e.g., *Semisulcospira* sp., *Bellamya* sp., *Cipangopaludina* sp., *Corbicula* sp. and *Limnoperna* sp.) were negatively correlated with the Cr, TP, HHCB and TCS concentrations, and occurred in higher abundances in the samples from the up- and midstream part of the Liuxi River (Fig. 5). All molluscs were positively correlated with the sand content, except for *Pomacea* sp., *Bithynia* sp. and *Stenothyra* sp. Almost no benthic macroinvertebrates were found in the samples from the central urban rivers (except for Sha River) which had higher levels of emerging chemicals, such as HHCB and TCS, in comparison with the Liuxi River and Zhujiang River.

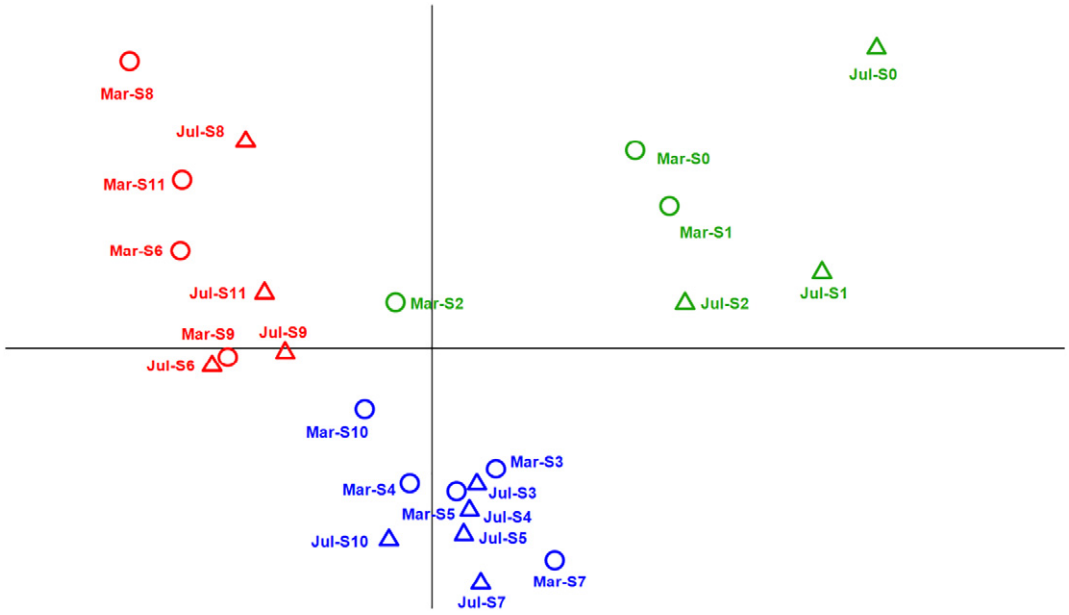


Fig. 4. Principal component analysis (PCA) ordination diagram performed on the bacterial OTU data for sediment samples from the six rivers of Guangzhou in the two seasons. The horizontal axis shows 23.1% of the variation between samples, the vertical another 14.9%. Circle and up triangle symbols represent samples from the dry and wet season, respectively. Green, blue and red symbols represent samples from the Liuxi River (S0–S2), downstream of the Liuxi River and Zhujiang River (S3–S5, S7 and S10), and central urban rivers (S6, S8, S9 and S11), respectively.

The benthic bacterial community composition at the OTU level was significantly correlated with HHCB, TP, TCS, Pb, and clay content (Fig. 6). Together they explained 46.8% of the total variance in bacterial community composition across samples (Fig. 6). HHCB, TP, TCS, Pb and clay content respectively explained 17.7%, 11.8%, 6.5%, 6% and 4.8% of the total variance. Among the 50 OTU's showing the most variation between samples, most of them occurred in higher abundances in the samples from central urban rivers relative to the Liuxi River and Zhujiang River (Fig. 6).

3.4. Correlation between macroinvertebrate traits and environmental variables

Only one trait (Reproduction: asexual reproduction) significantly modulated the species-to-environment relationship ($p < 0.05$), where the environment was represented by Cr and TP. Accordingly, only the first axis was significant. Cr and TP were positively correlated with the above trait (Fig. S3). When constrained by both the functional traits and environmental variables, 45% of the total variation in species data was explained. Furthermore, when constrained by either of them, environmental variables (i.e., Cr and TP) and the trait (i.e., Reproduction: asexual reproduction) explained comparable percentage of variation in species composition, i.e. 53% and 55%, respectively.

4. Discussion

4.1. Environmental variables in the sediment

The concentrations of heavy metals exceeded the toxic effect threshold (TET) values as set by the sediment quality guidelines for freshwater ecosystems at several sites (Table S3; MacDonald et al., 2000). For example, arsenic contamination was ubiquitous in the investigated rivers, with concentrations higher than the recommended TET value (17 μg/g dw) in 19 out of 24 samples. In addition, Cr, Cu, Hg and Ni were the four other metal contaminants with levels higher than the recommended TETs in more than half of samples. All the heavy metals concentrations were, however, below the TETs at sites S0 and S1, suggesting that the heavy metal contamination in the headwater of Liuxi River will not pose ecological risks. However, no guideline was available for the nutrients or the selected organic contaminants, except for 4-NP. Compared to the Canadian Sediment Quality Guideline (1.4 μg/g dw; Canada, 2002), most of the measured 4-NP concentrations exceeded this guideline value in both seasons.

4.2. Benthic community composition and spatio-temporal variation

4.2.1. Benthic macroinvertebrate communities

Among all the identified benthic macroinvertebrates, *L. hoffmeisteri*, which had absorber and deposit feeder as feeding habits, was the predominant macroinvertebrates at most sampling sites (except for S0, S1 and S8) where high levels of nutrients (mostly TP and TOC) and emerging organic compounds were determined (Dataset S1 and Fig. 5). This indicates that organically enriched habitats can be favourable conditions to recruit deposit-feeding organisms, as also demonstrated by Goodnight and Whitley (1960). Similar results have been reported in the Guangzhou Reach of the Zhujiang River (Sang, 1987) and in a heavily polluted stream (Jialu River) in eastern China (Wen et al., 2016), whose dominant taxa were L. *hoffmeisteri*, a typical pollution-tolerant species. In contrast, molluscs that had shredder, scraper, and/or filter-feeder as feeding habits mainly occurred at sites S0, S1 and S2 that were subjected to less environmental stress as compared to the remaining sites (Dataset S1 and Fig. 5). This is likely related to the different tolerance of various benthic macroinvertebrates to certain abiotic variables, such as oxygen level, substrate and the load of fine particulate organic matter (Doledec and Statzner, 2008). For instance, water samples from the sites S0, S1 and S2 had higher dissolved oxygen levels (4.14–6.86 mg/L) than those from the remaining sites (≤2.32 mg/L) (Table S1). These results suggest a shift of feeding habits from un-impacted (shredder, scraper, and/or filter-feeder) to impacted sites (absorber and deposit feeder), which is in agreement with the findings reported in the Nestore river basin (Central Italy; Pallottini et al., 2017). From the Liuxi River and Zhujiang River to the central urban rivers, a decrease in species diversity accompanied by an increase in emerging chemicals and nutrients suggests an effect of WWTP effluents on macrofaunal species assemblages (Table S1; Dataset S1 and S2). In contrast, an increasing trend in abundance of annelids (i.e., *L. hoffmeisteri* and *B. sowerbyi*) was found from the Liuxi River to the Zhujiang River. This is likely associated with the high total organic

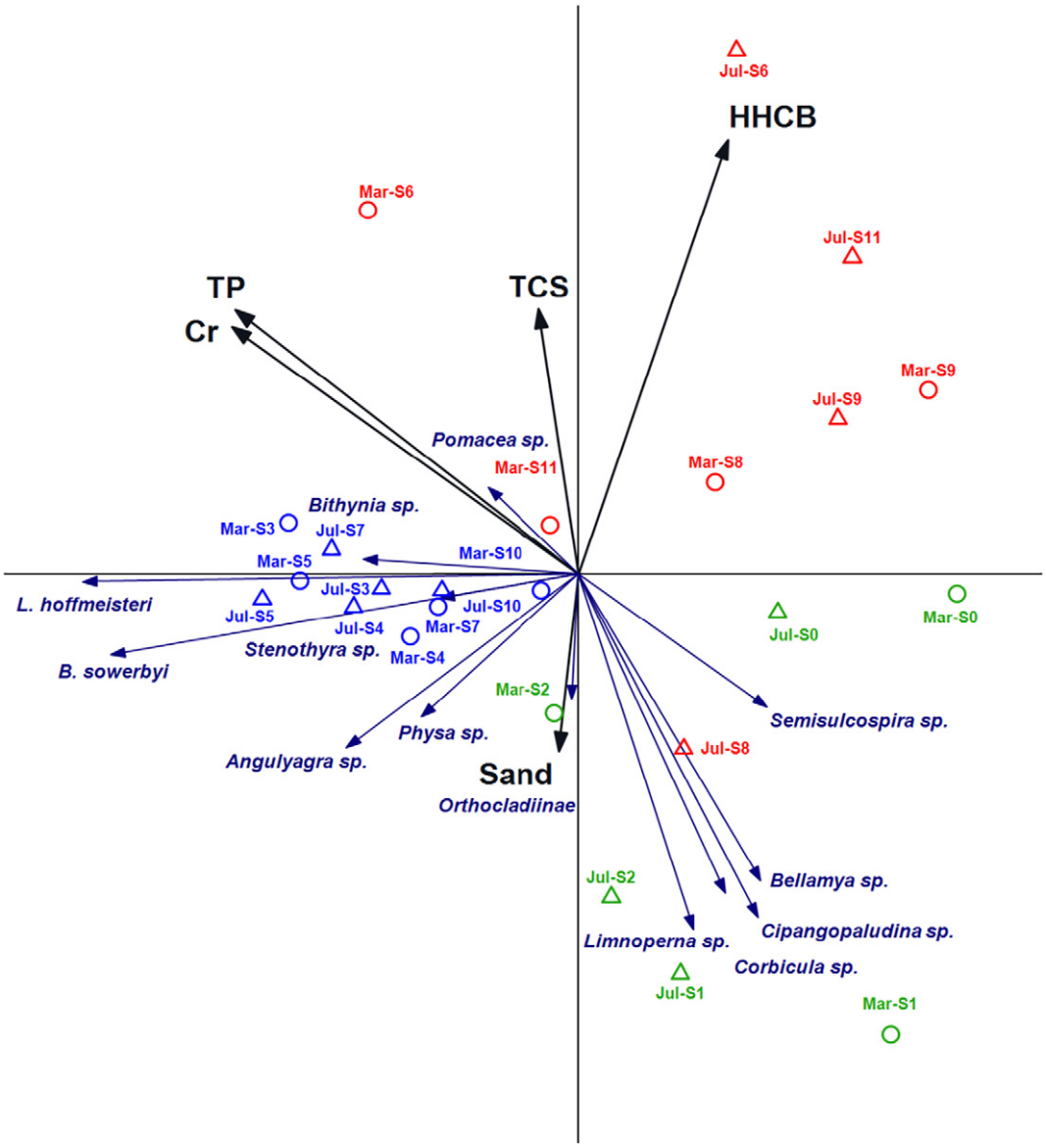


Fig. 5. Partial redundancy analysis (partial RDA) ordination diagram performed with the environmental variables that significantly explained the variation of benthic macroinvertebrate community composition as assessed by Monte Carlo permutation test. Of all the variance in invertebrate community composition observed in the dataset, 65.3% can be explained by the included environmental variables. The horizontal axis shows 52.6% of the variation between samples, the vertical another 8%. Circle and up triangle symbols represent samples from the dry and wet season, respectively. Green, blue and red symbols represent samples from the Liuxi River (S0–S2), downstream of the Liuxi River and Zhujiang River (S3–S5, S7 and S10), and central urban rivers (S6, S8, S9 and S11), respectively. Black arrows represent environmental variables, whereas blue arrows represent benthic macroinvertebrate taxa. Notably, no benthic macroinvertebrate was found in S9 in both seasons, S6 and S11 in the wet season. The *p* value was 0.002 for the Monte Carlo permutation test on all axes. For interpretation, see text.

matter and hypoxic conditions in the Zhujiang River and central urban rivers, as annelids can use organic matter for growth and are tolerant to hypoxic conditions (Omena et al., 2012). Similar findings have been reported for macroinvertebrates in the tidal flats of the Paranaguá Estuarine Complex (Brazil) subjected to different levels of contamination from urban effluents, whose dominant taxa were the tolerant organisms including *Tubificinae* sp1 (an annelid) (Souza et al., 2013). The drastic reductions in benthic macroinvertebrate diversity accompanying the presence of only a few opportunistic species (e.g., *Tubificidae*) with a high density (Dataset S1) suggests that rivers in the urban area of Guangzhou city were subjected to organic contamination (Bigot et al., 2008; Omena et al., 2012), which is most likely a result of WWTP effluents. This is also supported by the occurrence of emerging chemicals and nutrients measured in the sediment of these rivers, e.g. in both the dry and wet seasons the maximum concentration of 4-NP (14.4 and 11.7 μg/g dw, respectively) and TOC (144 and 97.3 mg/g dw, respectively) were determined at site S6 located downstream of a WWTP (Dataset S2).

4.2.2. Benthic bacterial communities

The benthic bacterial community composition shifted along the rural-urban gradient, which is in line with the findings observed in the urban and suburban rivers of Chicago which showed that anthropogenic activities (e.g., WWTP effluent discharges) altered the bacterial community composition directly and indirectly in the contaminated sites (Drury et al., 2013). For example, *Paracocccus*, *Zoogloea* and *Thauera* were prominent denitrifying genera in the activated sludge of WWTPs of California (USA; Tsai et al., 2012) and Henan Province (China; Wang et al., 2014), which were also observed at sites S6, S8 and S11 in our study (Table S2). This is likely related to the WWTP effluents and/or untreated wastewater discharge by the surrounding area. Since wastewater-associated bacteria *Acinetobacter*, *Arcobacter* and

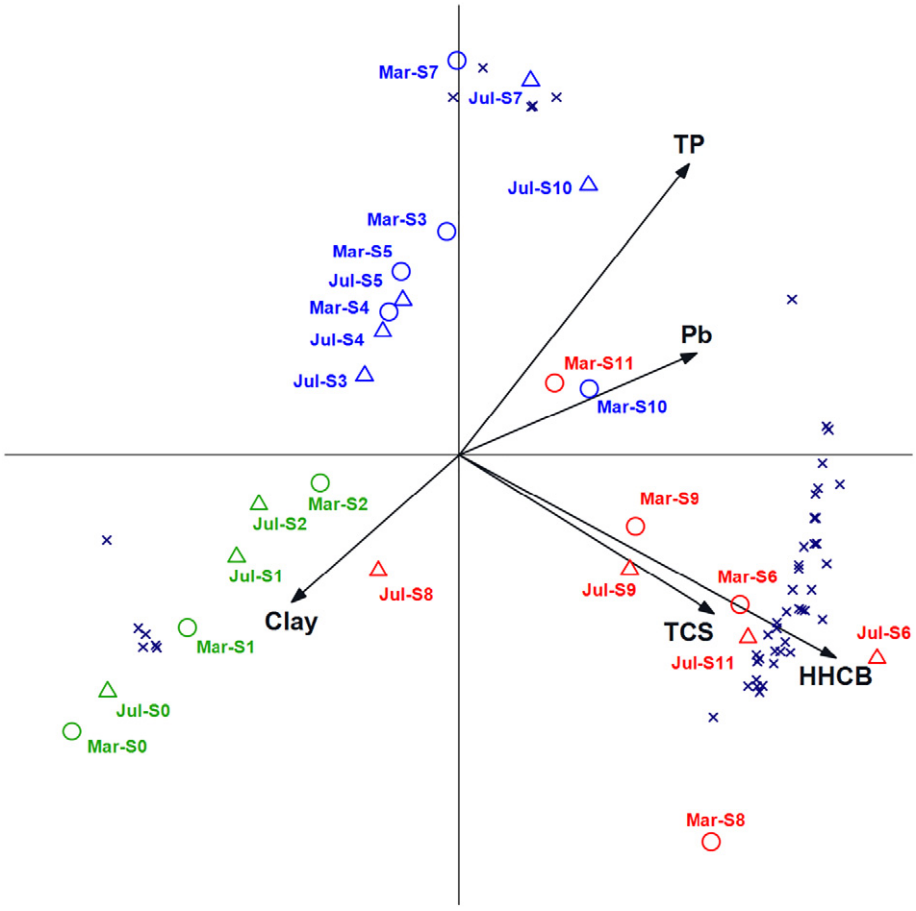


Fig. 6. Partial redundancy analysis (partial RDA) ordination diagram performed on the OTU table with the environmental variables that significantly explained the variation of the bacterial community composition as assessed by Monte Carlo permutation test. Of all the variance observed in the dataset, approximately 46.8% can be explained by the included variables. The horizontal axis shows 20% of the variation between samples, the vertical another 13.3%. Only the 50 OTU's for which the most variation is displayed in the diagram are shown. x symbols represent OTUs. Circle and up triangle symbols represent samples from the dry and wet season, respectively. Green, blue and red symbols represent samples from the Liuxi River (S0–S2), downstream of the Liuxi River and Zhujiang River (S3–S5, S7 and S10), and central urban rivers (S6, S8, S9 and S11), respectively. Black arrows represent environmental variables significantly explaining the variation of bacterial community composition across samples. The *p* value was 0.002 for the Monte Carlo permutation test on all axes. For interpretation, see text.

Trichococcus reached a high relative abundance at site S8 (Table S2), this site was likely more affected by WWTP effluents and/or untreated wastewater than the other sites. Indeed, there is a WWTP upstream of the Sha River, which routinely discharges effluents to the Sha River (Fig. 1). Additionally, TCC and TCS have been found in the effluent of this WWTP (45.6 and 35.0 ng/L, respectively), and the effluent discharges increased their concentrations in the sample (Sha River) approximately 100 m downstream from the outlet (from 14.2 to 92.8 ng/L for TCS and from 24.7 to 82.8 ng/L for TCC) (Liu et al., 2017). Although there is also a WWTP upstream of the Shijing River, the relative abundance of these wastewater-associated bacteria were lower at the site S6 (Shijing River) relative to the site S8 (Sha River). This is likely to be associated with the difference in the components of WWTP effluents between the two rivers, since the WWTP upstream of the Shijing River and Sha River employed different treatment technologies (modified anaerobic/anoxic/oxic (A^2/O) versus membrane bioreactor (MBR)), and the WWTP upstream of the Shijing River (maximum daily flow: 150,000 m^3/d; population served: 409,000; Table S1) is larger than that upstream of the Sha River (maximum daily flow: 100,000 m^3/d; population served: 130,000; Table S1). Additionally, the difference in untreated effluents could be another reason, as both rivers received a small proportion of untreated wastewater, e.g. runoff input and direct discharge of wastewater. Interestingly, chironomids were also more abundant at site S8 in the wet season, which may be a consequence of similarities in the preference for physicochemical environments between chironomids and those wastewater-associated bacteria. It is also possible that chironomids impacted the bacteria community composition at S8, as their biological activities, e.g. burrowing, irrigation (e.g., irrigation of burrows with overlying oxygen richer waster) and feeding, may alter both the physical structure, the oxygen content and the chemical equilibrium within the sediment and further affect the bacterial communities inhabiting the sediment (Laverock et al., 2010). In support of this, Peng et al. (2019b) found that the presence of benthic macroinvertebrates in the microcosms significantly increased the relative abundance of *Chloroflexi*, but it decreased the relative abundance of *Firmicutes* in the sediment. Additionally, it has been reported that the feeding activity of *Chironomus* larvae markedly decreased sediment bacterial abundance in laboratory experiments, probably as they use the bacteria as a food source (Johnson et al., 1989).

It should be noted that the bacterial community was clearly distinct between site S3 and the other sites from the Liuxi River (i.e., S0, S1 and S2) (Fig. 4). This is most likely related to the fact that the downstream part of the Liuxi River is located in the urban area of Guangzhou with higher levels of anthropogenic stress relative to the up- and midstream part (Peng et al., 2017). Despite the different environmental conditions (e.g., hydrological conditions, chemical and biological characteristics), the bacterial community at site S3 was similar to the sites S4 and S5 in the Zhujiang River (South China). Likewise, Drury et al. (2013) found that bacterial communities were clearly distinct at sites upstream of effluent input between the urban and suburban rivers in the Chicago metropolitan region, but they were indistinguishable at sites downstream of the WWTP effluents between rivers. These findings suggest that WWTP

effluents entering the environment may contribute to homogenization of the bacterial communities observed here. The seasonal variation in the sediment bacterial community composition was likely due to the higher temperature (Table S1; Wilcoxon matched-pairs test) and more inputs (due to runoff) in the wet season than the dry season.

4.3. Correlation between benthic community composition and environmental variables

We found that Cr, TP, HHCB and TCS together explained around 61% of the total variation in benthic macroinvertebrate composition among sites (Fig. 5). Similarly, Pb, TP, HHCB and TCS significantly explained the variation in the benthic bacterial community composition (Fig. 6). These results suggest that organic contamination caused by input of urban effluents might be a primary force in determining benthic community composition. For instance, the absence of benthic macroinvertebrates was observed in samples from the central urban rivers where high levels of nutrients were determined (Dataset S1 and S3). Correspondingly, the environmental risk assessment of selected emerging organic compounds in the sediments of these rivers showed that risk quotients (RQs) of TCS and 4-NP were larger than 1 in all samples from the four central urban rivers (Peng et al., 2017). Although the sand content explained about 5% of the total variation in the benthic macroinvertebrate composition, the explained fraction is lower than those of nutrients and organic contaminants. In contrast, Wolmarans et al. (2017) found that the sediment particle size played a key role in determining the distribution and abundance of benthic macroinvertebrate taxa in a minimally affected river in South Africa. Nevertheless, there were both minimally affected (the upstream part of the Liuxi River) and extensively contaminated rivers (the four urban rivers) in the present study, which may confound the effect of the sediment particle size on the occurrence of benthic macroinvertebrates. Likewise, there was a significant relationship between the bacterial community composition and sediment particle size, which is in agreement with the previous finding that particle size may be an important component of spatial heterogeneity in bacterial community structure (Jackson and Weeks, 2008). The unexplained variation in biological communities might be a result of other unmeasured environmental variables, such as water velocity and the presence of the reservoir. For example, it has been reported that the Yahagi-Daini Dam (Japan) severely altered macroinvertebrate assemblages at sampling sites located <1 km downstream (Takao et al., 2008). However, unlike that study, the reference sites (S1 and S2) in the present study are located >50 km downstream of the reservoir (Table S1), which would have reduced the impact of the presence of the reservoir above on benthic assemblages at these sampling sites. Overall, our results suggest that anthropogenic disturbance (i.e. environmental pollution) significantly contributed to the variation in benthic assemblages in rivers, even though we cannot rule out the influence of unmeasured ecological drivers.

4.4. Correlation between benthic macroinvertebrate traits and environmental variables

The most distinctive trait was asexual reproduction, suggesting that species changing along the environmental gradients also caused changes in the trait composition of the species assemblages, as demonstrated in a previous study of biological traits of soft bottom infaunal species assemblages in the Oslofjord (Norway), which has been severely influenced by municipal and industrial effluents (Oug et al., 2012). This is in line with the findings of a previous study investigating benthic macroinvertebrates in the Danube River where traits, including reproduction type, explained the difference in benthic macroinvertebrate composition among sampling sites (Rico et al., 2016). In the sites with higher levels of TP and Cr, the dominant traits were feeding habits (e.g., absorber and deposit feeder) and reproduction (e.g., asexual reproduction) (Datasets S1, S2 and S3; Fig. S3). Because asexual reproduction is an r-selected opportunistic trait (Jennings and Calow, 1975; Harriott, 1980), these results support the predictions that opportunistic traits are beneficial in disturbed areas (Bremner et al., 2006). It has been reported that the full trait information for each taxa may not be available and may further limit the power of biological traits analysis (Van den Brink et al., 2011; Munari, 2013). Nevertheless, this was not the case in our study, since the full trait information of each taxon was available in the Tachet database. Thus, this approach is useful to evaluate the effects of contamination on the benthic macroinvertebrate community in urban rivers, as traits can reflect the function of every species in a community (Bremner, 2008).

5. Conclusions

The results of this study show that both benthic macroinvertebrate- and bacterial community composition shifted along the rural-urban gradient. There were significant correlations between benthic communities and environmental variables (i.e., most analysed emerging chemicals, heavy metals and nutrients) in urban rivers. There was also a significant relationship between benthic community composition and sediment particle size. Absence of benthic macroinvertebrates was recorded at a few samples from the central urban rivers where high levels of nutrients and emerging organic compounds were determined. Our results suggest that anthropogenic activities (e.g., WWTP effluent discharges) altered the benthic community composition directly or indirectly in the contaminated sites.

Declaration of competing interest

All authors declare no conflict of interest.

Acknowledgments

The authors would like to acknowledge the financial support from the Research Institute for Fragrance Materials. We also thank the partial financial support from the National Natural Science Foundation of China (NSFC U1701242 and 41503108).

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.scitotenv.2019.136281.

References

Amato, K.R., Yeoman, C.J., Kent, A., Righini, N., Carbonero, F., Estrada, A., Gaskins, H.R., Stumpf, R.M., Yildirim, S., Torralba, M., Gillis, M., Wilson, B.A., Nelson, K.E., White, B.A., Leigh, S.R., 2013. Habitat degradation impacts black howler monkey (*Alouatta pigra*) gastrointestinal microbiomes. The Isme Journal 7, 1344–1353.

Atashgahi, S., Liebensteiner, M.G., Janssen, D.B., Smidt, H., Stams, A.J.M., Sipkema, D., 2018. Microbial synthesis and transformation of inorganic and organic chlorine compounds. Front. Microbiol. 9.

Bigot, L., Grémare, A., Amouroux, J.-M., Frouin, P., Maire, O., Gaertner, J.C., 2008. Assessment of the ecological quality status of soft-bottoms in Reunion Island (tropical Southwest Indian Ocean) using AZTI marine biotic indices. Mar. Pollut. Bull. 56, 704–722.

Bolam, S., Eggleton, J., 2014. Macrofaunal production and biological traits: spatial relationships along the UK continental shelf. J. Sea Res. 88, 47–58.

Boothroyd, I., Stark, J., 2000. Use of Invertebrates in Monitoring. New Zealand Stream Invertebrates: Ecology and Implications for Management. pp. 344–373.

Bremner, J., 2008. Species' traits and ecological functioning in marine conservation and management. J. Exp. Mar. Biol. Ecol. 366, 37–47.

Bremner, J., Rogers, S.I., Frid, C.L.J., 2006. Matching biological traits to environmental conditions in marine benthic ecosystems. J. Mar. Syst. 60, 302–316.

Burton, J.A.G., 2002. Sediment quality criteria in use around the world. Limnology 3, 65–76.

Canada, 2002. Canadian Environmental Quality Guidelines. Canadian Council of Ministers of the Environment.

Cheung, K.C., Poon, B.H.T., Lan, C.Y., Wong, M.H., 2003. Assessment of metal and nutrient concentrations in river water and sediment collected from the cities in the Pearl River Delta, South China. Chemosphere 52, 1431–1440.

Culp, J.M., Armanini, D.G., Dunbar, M.J., Orlofske, J.M., Poff, N.L., Pollard, A.I., Yates, A.G., Hose, G.C., 2011. Incorporating traits in aquatic biomonitoring to enhance causal diagnosis and prediction. Integr. Environ. Assess. Manag. 7, 187–197.
Doledec, S., Statzner, B., 2008. Invertebrate traits for the biomonitoring of large European rivers: an assessment of specific types of human impact. Freshw. Biol. 53, 617–634.
Drury, B., Rosi-Marshall, E., Kelly, J.J., 2013. Wastewater treatment effluent reduces the abundance and diversity of benthic bacteria communities in urban and suburban Rivers. Appl. Environ. Microbiol. 79, 1897–1905.
Edgar, R.C., Haas, B.J., Clemente, J.C., Quince, C., Knight, R., 2011. UCHIME improves sensitivity and speed of chimera detection. Bioinformatics 27, 2194–2200.
Goodnight, C.J., Whitley, L.S., 1960. Oligochaetes as indicators of pollutions. Wat. & Sewage Wks 107, 311.
Harriott, V.J., 1980. The Ecology of Holothurian Fauna of Heron Reef and Moreton Bay. MSc Thesis. The University of Queensland, Brisbane, Australia.
Ibekwe, A.M., Ma, J., Murinda, S.E., 2016. Bacteria community composition and structure in an Urban River impacted by different pollutant sources. Sci. Total Environ. 566–567, 1176–1185.
Jackson, C.R., Weeks, A.Q., 2008. Influence of particle size on bacterial community structure in aquatic sediments as revealed by 16S rRNA gene sequence analysis. Appl. Environ. Microbiol. 74, 5237–5240.
Jennings, J., Calow, P., 1975. The relationship between high fecundity and the evolution of entoparasitism. Oecologia 21, 109–115.
Johnson, R.K., Boström, B., van de Bund, W., 1989. Interactions between *Chironomus plumosus* (L.) and the microbial community in surficial sediments of a shallow, eutrophic lake. Limnol. Oceanogr. 34, 992–1003.
Jordan, S.J., Smith, L.M., 2005. Indicators of ecosystem integrity for estuaries. In: Bortone, S.A. (Ed.), Estuarine Indicators. CRC Press, Florida, USA, pp. 467–480.
Laverock, B., Smith, C.J., Tait, K., Osborn, A.M., Widdicombe, S., Gilbert, J.A., 2010. Bioturbating shrimp alter the structure and diversity of bacterial communities in coastal marine sediments. The ISME journal 4, 1531.
Li, D., Sharp, J.O., Drewes, J.E., 2016. Influence of wastewater discharge on the metabolic potential of the microbial community in river sediments. Microb. Ecol. 71, 78–86.
Liu, B., Hu, K., Jiang, Z., Yang, J., Luo, X., Liu, A., 2011. Distribution and enrichment of heavy heavy metals in a sediment core from the Pearl River Estuary. Environ. Earth Sci. 62, 265–275.
Liu, W.-R., Yang, Y.-Y., Liu, Y.-S., Zhang, L.-J., Zhao, J.-L., Zhang, Q.-Q., Zhang, M., Zhang, J.-N., Jiang, Y.-X., Ying, G.-G., 2017. Biocides in wastewater treatment plants: mass balance analysis and pollution load estimation. J. Hazard. Mater. 329, 310–320.
MacDonald, D.D., Ingersoll, C.G., Berger, T.A., 2000. Development and evaluation of consensus-based sediment quality guidelines for freshwater ecosystems. Arch. Environ. Contam. Toxicol. 39, 20–31.
Martino, D.P., Grossman, E.L., Ulrich, G.A., Burger, K.C., Schlichenmeyer, J.L., Suflita, J.M., Ammerman, J.W., 1998. Microbial abundance and activity in a low-conductivity aquifer system in east-central Texas. Microb. Ecol. 35, 224–234.
Moreno, P., Callisto, M., 2006. Benthic macroinvertebrates in the watershed of an urban reservoir in southeastern Brazil. Hydrobiologia 560, 311–321.
Munari, C., 2013. Benthic community and biological trait composition in respect to artificial coastal defence structures: a study case in the northern Adriatic Sea. Mar. Environ. Res. 90, 47–54.
Newton, R.J., Bootsma, M.J., Morrison, H.G., Sogin, M.L., McLellan, S.L., 2013. A microbial signature approach to identify fecal pollution in the waters off an urbanized coast of Lake Michigan. Microb. Ecol. 65, 1011–1023.
Omena, E.P., Lavrado, H.P., Paranhos, R., Silva, T.A., 2012. Spatial distribution of intertidal sandy beach polychaeta along an estuarine and morphodynamic gradient in an eutrophic tropical bay. Mar. Pollut. Bull. 64, 1861–1873.
Oug, E., Fleddum, A., Rygg, B., Olsgard, F., 2012. Biological traits analyses in the study of pollution gradients and ecological functioning of marine soft bottom species assemblages in a fjord ecosystem. J. Exp. Mar. Biol. Ecol. 432, 94–105.
Pallottini, M., Cappelletti, D., Fabrizi, A., Gaino, E., Goretti, E., Selvaggi, R., Céréghino, R., 2017. Macroinvertebrate functional trait responses to chemical pollution in agricultural–industrial landscapes. River Res. Appl. 33, 505–513.
Peng, F.J., Pan, C.G., Zhang, M., Zhang, N.S., Windfeld, R., Salvito, D., Selck, H., Van den Brink, P.J., Ying, G.G., 2017. Occurrence and ecological risk assessment of emerging organic chemicals in urban rivers: Guangzhou as a case study in China. Sci. Total Environ. 589, 46–55.
Peng, F.J., Diepens, N.J., Pan, C.G., Ying, G.G., Salvito, D., Selck, H., Van den Brink, P.J., 2019a. Response of sediment bacterial community to triclosan in subtropical freshwater benthic microcosms. Environ. Pollut. 248, 676–683.
Peng, F.J., Kiggen, F., Pan, C.G., Bracewell, S.A., Ying, G.G., Salvito, D., Selck, H., Van den Brink, P.J., 2019b. Fate and effects of sediment-associated polycyclic musk HHCB in subtropical freshwater microcosms. Ecotoxicol. Environ. Saf. 169, 902–910.
Reid, A.J., Carlson, A.K., Creed, I.F., Eliason, E.J., Gell, P.A., Johnson, P.T.J., et al., 2019. Emerging threats and persistent conservation challenges for freshwater biodiversity. Biol. Rev. 94, 849–873.
Rico, A., Van den Brink, P.J., Leitner, P., Graf, W., Focks, A., 2016. Relative influence of chemical and non-chemical stressors on invertebrate communities: a case study in the Danube River. Sci. Total Environ. 571, 1370–1382.
Sang, Q., 1987. Some ecological aspects of aquatic oligochaetes in the Lower Pearl River (People's Republic of China). Hydrobiologia 155, 199–208.
Schloss, P.D., Westcott, S.L., Ryabin, T., Hall, J.R., Hartmann, M., Hollister, E.B., Lesniewski, R.A., Oakley, B.B., Parks, D.H., Robinson, C.J., Sahl, J.W., Stres, B., Thallinger, G.G., Van Horn, D.J., Weber, C.F., 2009. Introducing mothur: open-source, platform-independent, community-supported software for describing and comparing microbial communities. Appl. Environ. Microbiol. 75, 7537–7541.
Souza, F., Brauko, K., Lana, P., Muniz, P., Camargo, M., 2013. The effect of urban sewage on benthic macroinvertebrates: a multiple spatial scale approach. Mar. Pollut. Bull. 67, 234–240.
Souza, F., Brauko, K., Gilbert, E., Martins, C., Lana, P., Camargo, M., 2016. Complex spatial and temporal variation of subtropical benthic macroinvertebrates under sewage impact. Mar. Environ. Res. 116, 61–70.
Staley, C., Gould, T.J., Wang, P., Phillips, J., Cotner, J.B., Sadowsky, M.J., 2014. Bacteria community structure is indicative of chemical inputs in the Upper Mississippi River. Front. Microbiol. 5, 524.
Staley, C., Gould, T.J., Wang, P., Phillips, J., Cotner, J.B., Sadowsky, M.J., 2015. Species sorting and seasonal dynamics primarily shape bacteria communities in the Upper Mississippi River. Sci. Total Environ. 505, 435–445.
Statzner, B., Beche, L.A., 2010. Can biological invertebrate traits resolve effects of multiple stressors on running water ecosystems? Freshw. Biol. 55, 80–119.
Tachet, H., Richoux, P., Bournaud, M., Usseglio-Polatera, P., 2000. Invertébrés d'eau douce: systématique, biologie, écologie. CNRS Editions, Paris, France.
Takao, A., Kawaguchi, Y., Minagawa, T., Kayaba, Y., Morimoto, Y., 2008. The relationships between benthic macroinvertebrates and biotic and abiotic environmental characteristics downstream of the Yahagi Dam, Central Japan, and the state change caused by inflow from a tributary. River Res. Appl. 24, 580–597.
Ter Braak, C.J., Šmilauer, P., 2018. Canoco Reference Manual and User's Guide: Software for Ordination (Version 5.1). Microcomputer Power, Ithaca, USA (536 pp).
Ter Braak, C.J., Šmilauer, P., Dray, S., 2018. Algorithms and biplots for double constrained correspondence analysis. Environ. Ecol. Stat. 1–27.
Tsai, C.Y., TongZhou, W., Asvapathanagul, P., Gedalanga, P.B., Hayden, D., La, J., Olson, B.H., 2012. Thauera spp., another prominent denitrifying bacteria in the methanol-fed activated sludge. Proc. Water Environ. Fed. 2012, 3718–3728.
Usseglio-Polatera, P., Thomas, S., Beisel, J.N., Moreteau, J.C., 1999. Biological trait structure of macroinvertebrate benthic communities. Illustration de la valeur indicatrice des caracteristiques biologiques des macroinvertebres d'une communaute benthique a differentes echellesd'observation. Ann. Limnol. 35, 71–80.
Usseglio-Polatera, P., Bournaud, M., Richoux, P., Tachet, H., 2000. Biological and ecological traits of benthic freshwater macroinvertebrates: relationships and definition of groups with similar traits. Freshw. Biol. 43, 175–205.
Van den Brink, P.J., Alexander, A.C., Desrosiers, M., Goedkoop, W., Goethals, P.L.M., Liess, M., Dyer, S.D., 2011. Traits-based approaches in bioassessment and ecological risk assessment: strengths, weaknesses, opportunities and threats. Integr. Environ. Assess. Manag. 7, 198–208.
VandeWalle, J.L., Goetz, G.W., Huse, S.M., Morrison, H.G., Sogin, M.L., Hoffmann, R.G., Yan, K., McLellan, S.L., 2012. Acinetobacter, Aeromonas and Trichococcus populations dominate the microbial community within urban sewer infrastructure. Environ. Microbiol. 14, 2538–2552.
Wang, Z., Zhang, X.X., Lu, X., Liu, B., Li, Y., Long, C., Li, A., 2014. Abundance and diversity of bacterial nitrifiers and denitrifiers and their functional genes in tannery wastewater treatment plants revealed by high-throughput sequencing. PLoS One 9, e113603.
Wang, L., Zhang, J., Li, H.-L., Yang, H., Peng, C., Peng, Z.-S., Lu, L., 2018. Shift in the microbial community composition of surface water and sediment along an urban river. Sci. Total Environ. 627, 600–612.
Wen, T., Sheng, S., An, S., 2016. Relationships between stream ecosystem properties and landscape composition at multiple spatial scales along a heavily polluted stream in China: implications for restoration. Ecol. Eng. 97, 493–502.
Wolmarans, C.T., Kemp, M., de Kock, K.N., Wepener, V., 2017. The possible association between selected sediment characteristics and the occurrence of benthic macroinvertebrates in a minimally affected river in South Africa. Chem. Ecol. 33, 18–33.